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PRINCIPAL INVESTIGATOR: Yi Huang, M.D., Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine
Baltimore, MD 21231

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Yi Huang, M.D., Ph.D.

Email: yhuang19@jhmi.edu

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14. ABSTRACT

Naure polyamines play an important role in cell proliferation and differentiation. Synthetic polyamine analogues can mimic natural polyamines in the down-regulation of polyamine biosynthesis, but analogues cannot promote cell growth. Our previous results show that polyamine analogues also down-regulate estrogen receptor α (ERα), the principle target in human breast cancer therapy. This proposal was designed to investigate the molecular mechanisms and the therapeutic efficacy of oligomines in the treatment of human breast cancer. In the fourth year of this award, we investigated the possible roles of the polyamines biosynthetic pathway in polyamine analogues mediated repress on ERα. In our latest studies, we demonstrated that ERα expression was repressed after ornithine decarboxylase (ODC), the polyamines biosynthetic key enzyme, was down-regulated by siRNA in several human breast cancer cell lines. Apoptosis and cell cycle arrest were also induced. These results indicated that the polyamine synthetic pathway is a key mediator of polyamine analogue induced ER alpha suppression.

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Polyamine analogues, breast cancer, growth inhibition, apoptosis.

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Introduction……...4

Body……4

Key Research Accomplishments……5

Conclusions……..5

References……7

Introduction

The critical role of polyamines in cell growth has led to the development of a number of strategies to interfere with polyamine metabolism including the novel polyamine analogues known as oligoamines. Our previous studies showed that the oligoamines significantly inhibit the key polyamine biosynthesis enzyme, ornithine decarboxylase and decrease the polyamine pools. The most importantly, our data demonstrated that oligoamines suppress expression and the ligand-dependent transcriptional activity of the estrogen receptor α (ERα), a principal determinant of breast cell growth and therapy. However, the mechanism of how oligoamines suppression of ERα is still unclear.

The purpose of this project is to elucidate the molecular mechanisms and the therapeutic efficacy of a novel class of polyamine analogues in the treatment of human breast cancer.

Body

Technical Objective 1: To determine the role of the polyamine biosythetic pathway in ER suppression by polyamine analogues.

In our previous results, we have demonstrated that CGC-11144 and several other polyamine analogues have suppressed the mRNA transcript and protein expression of estrogen receptor α in human breast cancer cells, whereas neither ERbeta nor other steroid hormonal receptors are affected by oligoamines. The possible mechanism of suppression of estrogen receptor α have been investigated.

To investigate whether the down-regulation of ERα by oligoamines occurs through the down-regulation of polyamine biosyntheses, siRNA interfering experiments were carried out. Two specific siRNAs were designed and synthesized. We demonstrated that we could suppress the ornithine decarboxylase gene transcript and significantly decrease the protein level of ornithine decarboxylase by siRNA (Fig. 1). Subsequently, polyamine pool level was decreased after siRNA treatment (Fig. 2).

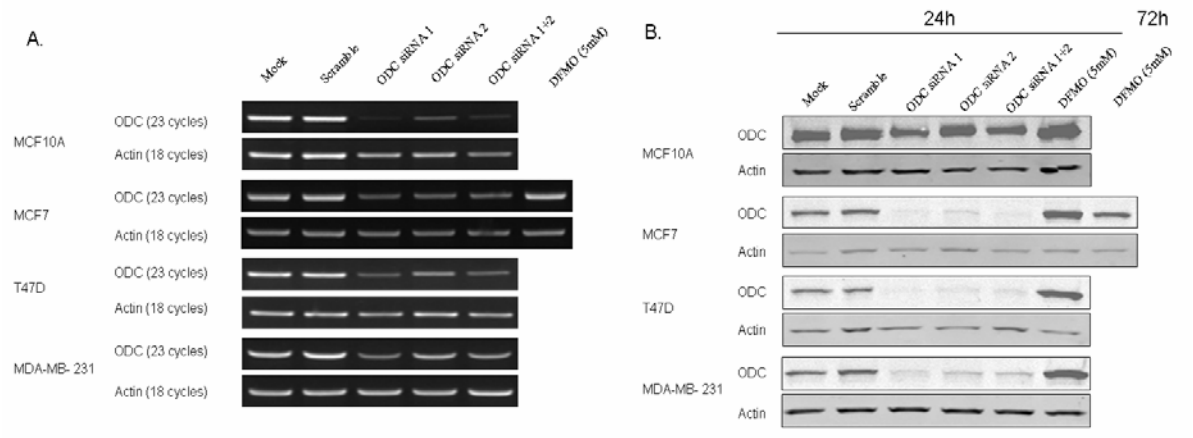

Fig. 1. Effects of siRNA on ornithine decarboxylase transcripts and proteins. Breast cell lines were transient transfected by ODC siRNA or treated by 5 μ M DFMO, a ornithine decarboxylase inhibitor. 24h after transfection, total RNA were prepared for RT-PCR to measure ODC transcript levels (A) or total proteins were prepared for Western blotting to measure ODC protein level (B).

We also observed that ERα mRNA and proteins were markedly decreased by ornithine decarboxylase siRNA in MCF7 and T47D cells, whereas other steroid hormone receptors, RAR,

and VDR, were unaffected by ODC siRNA (Fig.3). Subsequently, decreased expression of progesterone receptor (PR) and cyclin D1, two downstream estrogen receptor-regualted genes was also observed. We further demonstrated that down-regulation of ODC led cell cycle arrest and increased apoptosis. ODC siRNAs were transit transfected MCF7 cells. Cell growth was inhibited and PARP cleavage was observed(Fig. 4).

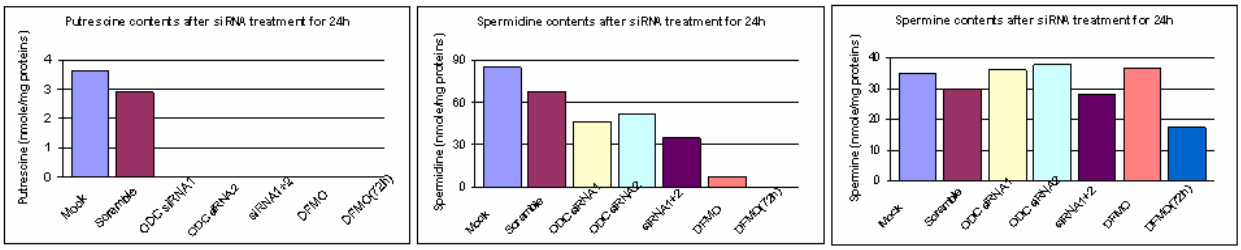

Fig. 2. Regulation of intracellular polyamine pools by ornithine decarboxylase siRNA. Polyamine pools (putrescine, spermidine and spermine) were assessed in MCF7 cells after the cells were transiently transfected with ornithine decarboxylase siRNA.

Technical Objective 2: Investigate the molecular mechanisms by which polyamine analogs repress ER gene transcription

DNA affinity precipitation assays (DAPA) and mass spectrometry was performed to identify and monitor the recruitment of transcription factors at the ER minimal promoter. Biotin-labeled oligonucleotides were designed and synthesized. The components of the multi-proteins complexes bound to ER minimal promoter could be precipitated and identified. The results of this study are currently under investigating and evaluation.

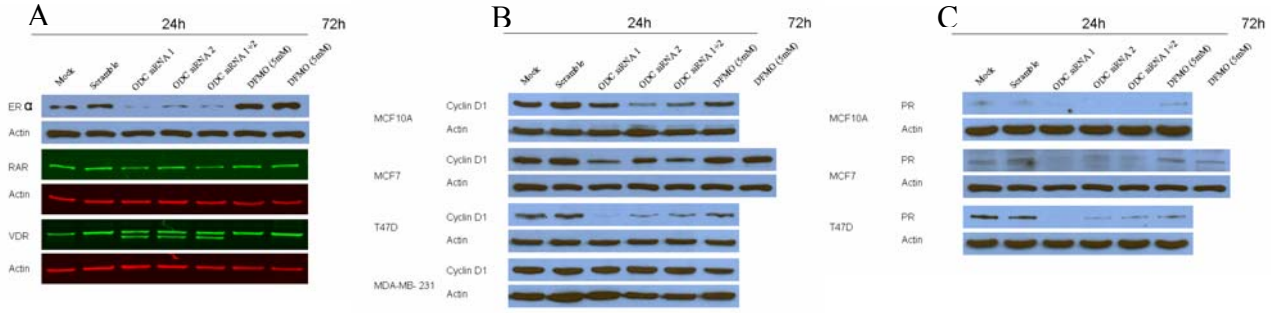

Fig. 3. Effects of ornithine decarboxylase siRNA on ERα and ERα-responsive genes. Ornithine decarboxylase siRNA was specifically suppressed estrogen receptor α (ERα), but not other steroid hormonal receptors (A). ERα-responsive genes, cyclin D1 (B) and progestron receptor (PR) (C) were down-regulated by ODC siRNA.

Key Research Accomplishments

1) Our studies suggest that the polyamine biosynthetic pathway plays an integral role in oligoamine mediated down-regulation of ERα.
2) Interference of polyamine biosynthesis contributes to oligoamine-induced cell cycle arrest and apoptosis in human breast cancer cells.

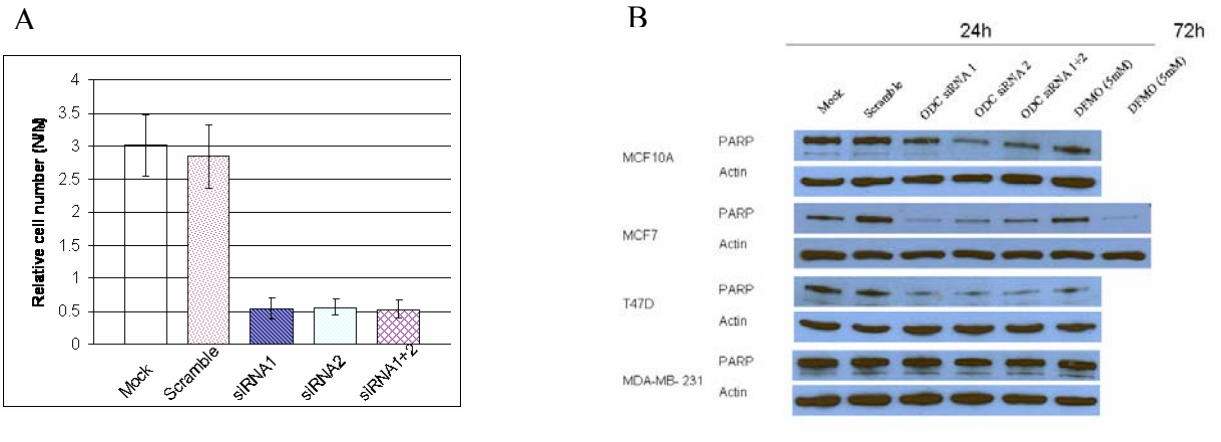

Fig.4. Effects of ornithine decarboxylase siRNA on cell growth and apoptosis. Cell growth was inhibited by ornithine decarboxylase siRNA in MCF7 cells (A) and apoptosis was induced (B).

Conclusions

Intracellular polyamines are essential for cell growth. Polyamine analogues can mimic natural polyamine regulation but are biologically inactive or have altered functions. An innovative class of polyamine analogues, oligoamines has been developed for cancer treatment. The oligoamines down-regulate polyamine biosynthesis and inhibit breast cancer cell growth by induction of apoptosis. They specifically suppress expression and activity of the estrogen receptor alpha (ERα), a principal determinant of growth and differentiation in human breast cancer cells. We have demonstrated that down-regulation of ornithine decarboxylase by siRNA suppresses the protein expression of ornithine decarboxylase and decreases the cellular polyamine pools. Expression of ERα, and downstream ERα responsive genes are repressed. These findings also suggest that the polyamine biosynthetic pathway has an important role in the down-regulation of ERα expression by polyamine analogues in breast cancer cells and underscored the rationale of targeting the polyamine biosynthetic pathway as a potential approach to breast cancer therapy and/or prevention.

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